EFFECT OF TENSILE STRESS ON THE ULTRASTRUCTURE
OF BOVINE MUSCLE

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──────────── ABSTRACT ────────────

The changes in ultrastructure of bovine semitendinosus, both raw and heated (90°C) and subjected to tensile stress, were determined by scanning electron microscopy. Stress parallel to the fiber axis resulted in the initial rupture of the muscle fiber-endomysium sheath. Perpendicular stress caused initial rupture at the endomysium-perimysium junction with the muscle fibers remaining undisturbed. Similar observations were made on the muscle tissue heated to 90°C and subjected to tensile stress. Heating to 90°C does not alter completely the fibrous nature of the perimysium connective tissue. Some native type collagen fibrils were observed, even after the severe heat treatment. The effect of applied stress on components of the muscle tissue is an important consideration in the development of a more reliable test method for evaluation of meat tenderness.

INTRODUCTION

PROBABLY the most important factor influencing meat quality, apart from wholesomeness, is tenderness. The literature contains a large number of papers related to the identification of those factors contributing to meat tenderness. Some of these considerations include: sarcomere length (Bouton et al., 1974; Hegarty and Allen, 1975; Herring et al., 1965); pH (Hamm and Deatherage, 1960); connective tissue (Dutson, 1974; Paul et al., 1973); age (Bouton and Harris, 1972); cooking temperature (Bouton et al., 1974; Hegarty and Allen, 1975; Jones et al., 1977; Schaller and Powrie, 1972; Schmidt and Parrish, 1971); method of hanging (Herring et al., 1965); location of muscle (Herring et al., 1965); and many others. Laakkonen (1973) in an excellent review paper has examined many of these factors.

Subjective evaluation of meat tenderness is best determined by use of a trained taste panel, but it is expensive and time consuming and gives results after the fact. Pearson (1963) reviewed methods for the objective determination of meat tenderness. He noted that the most widely used is the Warner-Bratzler shear test, but that the correlation of this with sensory methods is poor, averaging about 0.75 (range 0.60–0.85). An analysis of this test indicates that tensile, compression, and shear forces are involved with the measurement.

Stanley et al. (1972) attempted to correlate tensile stress with sensory evaluation of meat tenderness, but the coefficient of variation ranged up to 54%. L'Hirondelle and Martin (1975) reported that an attempt to estimate tenderness of raw and cooked beef muscle by comparison of various objective methods with a trained sensory panel proved unsatisfactory. Herring (1976) discussed some of the more important factors related to meat texture and the instrumental methods used to evaluate tenderness. He classified the instruments according to the principal action: shearing, biting, penetrating, compressing and stretching, and breaking.

In our study, we attempted to isolate a single stress factor, tensile, and determine its effect on the muscle fiber and the connective tissue structure. To carry out this work, we

developed a reproducible sample preparatory procedure (Jones et al., 1976) and studied the effects of heating at various temperatures on bovine muscle structure (Jones et al., 1977).

With the techniques of light and electron microscopy, raw and heated tissue samples are characterized to determine the effects of applied tensile stress on the meat system. Structural information of this nature hopefully can lead to a better understanding of these effects and eventually to the development of more precise objective methods for predicting meat tenderness.

MATERIALS & METHODS

Sample source

The bovine semitendinosus muscle (eye of round) was obtained either commercially (ten samples) or from a carcass obtained at slaughter and aged in a cold room maintained at 0.5°C for 10 days. Results obtained with muscle from the two sources were indistinguishable.

Heating

For the heating experiments, the tissue was immersed in H_2O in a polyethylene bag and heated in a water bath at 90°C. The sample required 15–20 min to reach equilibrium temperature, as monitored by a thermocouple inserted in the sample and was held at that temperature for 45 min.

Stressing

The semitendinosus muscle was cut parallel to the fiber axis into slices about 6 mm thick. Pieces 6 mm × 20 mm were cut from the large slice for the stress experiments. Raw and heated muscle samples were stressed either parallel or perpendicularly to the muscle fiber axis to various extensions up to and including break. Both raw and heated muscle tissue were evaluated at (a) rest length-control, (b) stressed parallel to fiber axis, and (c) stressed perpendicularly to fiber axis.

Initial stress experiments were carried out as described by Stanley et al. (1972) on an Instron Universal testing apparatus. For carrying out dynamic stressing studies of muscle tissue, a minitensile stage (Fig. 1a) was designed and built. For more general application, this stage was designed to operate in the specimen chamber of the scanning electron microscope. All stress experiments were performed on hydrated specimens and observed under a Bausch and Lomb Stereo 7 zoom microscope at magnifications up to 200X. These experiments were recorded at the appropriate magnification with a GBC-ITC 5000 video camera coupled to the stereo microscope and recorded on an Ampex 5200 video tape deck. Video tape observations were compared to specific areas observed in the scanning electron microscope (SEM).

Handling of stressed tissue

We designed and built several special devices in order to fix and observe the tissue in the stressed position. An adjustable clamping device (Fig. 1b) consisting of two hemostats (one fixed and one variable) permitted fixation of the sample under stress. The sample was transferred to a clamping holder (Fig. 1c) designed to hold the tissue in a stressed position and fit in the specimen holder of the scanning electron microscope. Thus, manipulation of the specimen was minimized. Identical areas of the specimen could be observed and compared in both the light and electron microscopes.

Sample preparation

All muscle samples were restrained and fixed overnight in a modified aldehyde fixative (Jones et al., 1976). The fixative contained 2% glutaraldehyde and 2% paraformaldehyde in 0.05M phosphate buffer. The pH of the fixative was adjusted to the pH of the particular muscle sample which, at 10–14 days postmortem, was 5.6–5.9. The samples were thoroughly washed with water, dehydrated through increasing ethanol water series of 70%, 95% (2X), and 100% (2X). At this point the interior surfaces of the tissue, control or stressed, were exposed for

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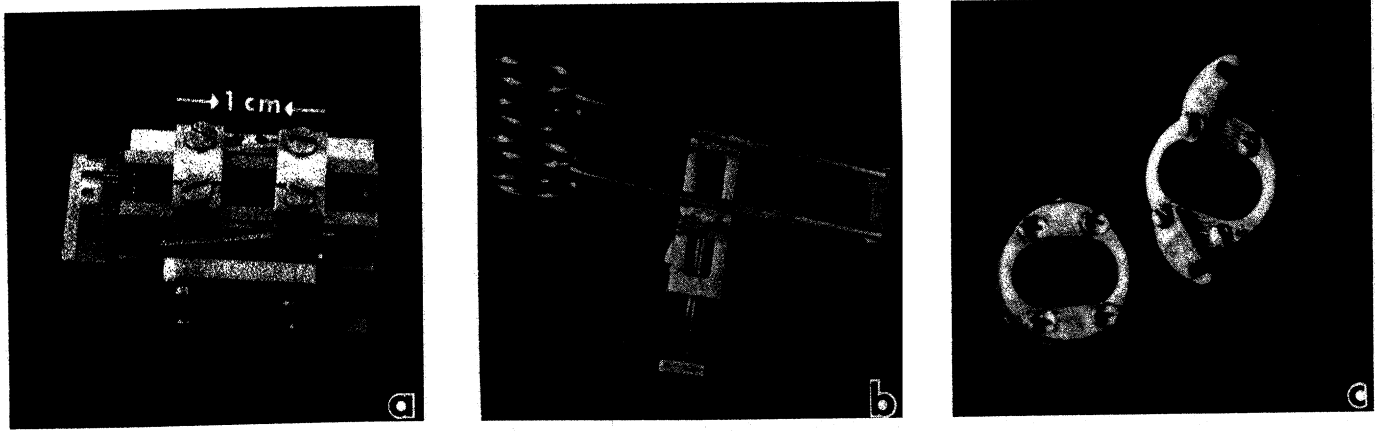
Fig. 1—Mechanical devices used in muscle studies: (a) minitensile stage for stressing tissue; (b) tissue clamping device; (c) tissue clamping holder for SEM.

observation by the cryofracture technique (Humphreys et al., 1974). This procedure involves freezing the sample in ethanol at liquid nitrogen temperature and fracturing the samples while frozen. By this method interior surfaces are obtained which have not been mechanically damaged. The fractured samples were critical point dried with liquid carbon dioxide as a transitional fluid and then mounted on specimen stubs with silver paint. A layer of carbon, followed by gold-palladium, was deposited to minimize charging effects.

Isolation of perimysium

Slices of semitendinosus, approximately 6 mm thick, were cut perpendicularly to the fiber axis. Slices were heated at 90°C as previously described. Samples of perimysial connective tissue from both raw and heated slices were removed by careful dissection under a stereo microscope by use of a scalpel to separate the connective tissue from the muscle fibers. The separated perimysium was fixed in glutaraldehyde, dehydrated, critical point dried, and mounted on stubs for observation on scanning electron microscopy. Portions of the fixed

perimysium were dispersed in H_2O in a Waring Blendor and deposited on 200 mesh grids for observation in the transmission electron microscope.

Electron microscopy

A JEOL 50-A scanning electron microscope (SEM) operating at 10–15 kV and an RCA EMU 3-G transmission electron microscope (TEM) operating at 100 kV were used in this investigation.

RESULTS

SCANNING ELECTRON MICROGRAPHS of raw muscle tissue fractured perpendicularly and parallel to the fiber axis are shown in Figure 2. These illustrate the relationships of the various components of the muscle system. As shown in Figure 2a (perpendicular fracture), the perimysium (P) is composed of a random network of fibers which separate muscle fiber
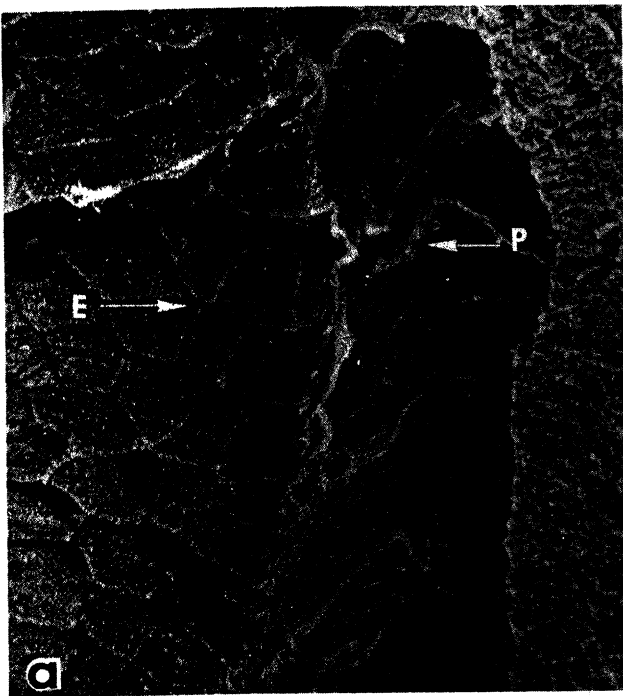

Fig. 2(a)—Raw semitendinosus fractured perpendicularly to fiber axis. The fibers are loosely surrounded by endomysium (E). The perimysium (P) with thicker connective tissue fibers enclose the muscle fiber bundles.
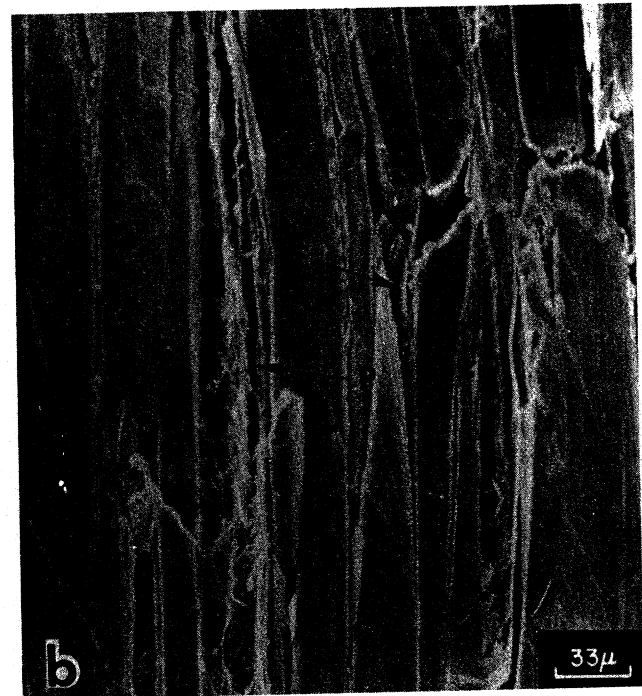

Fig. 2(b)—Raw semitendinosus fractured parallel to fiber axis. Endomysium (E) and perimysium (P) connective tissues are shown.
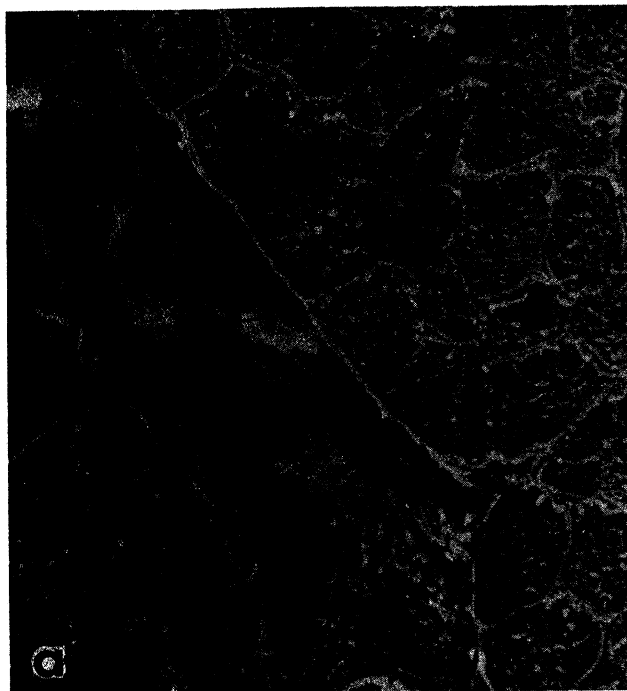
Fig. 3(a)—Heated (90°C) semitendinosus fractured perpendicularly to fiber axis. Endomysium (E) and muscle fibers are in close contact. The perimysium, while still fibrous, contains amorphous material.
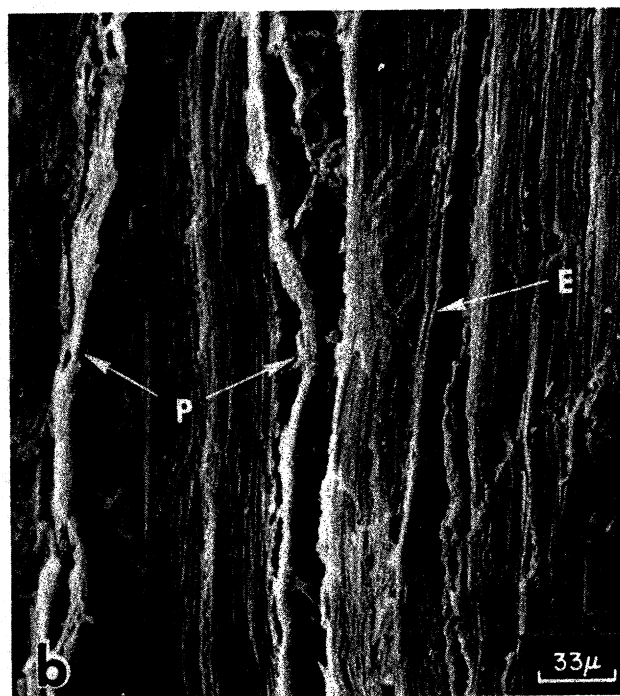

Fig. 3(b)—Heated (90°C) semitendinosus fractured parallel to fiber axis. The endomysium (E) is congealed. Perimysium (P) can be seen running down both sides of a fiber bundle. The fibrils making up the fiber have been exposed by the fracture.

bundles. The fiber bundle is composed of individual muscle fibers, each surrounded by the endomysium sheath (E). This sheath is internally connected within the fiber bundle and coalesces at the periphery of the bundle with the coarse fibers of the perimysium. In Figure 2b, muscle tissue fractured parallel to the fiber axis is observed. The perimysium (P) and the endomysium connective tissue (E) are clearly discernible.

This organizational structure is still evident in cooked samples (Fig. 3), although a number of significant changes have taken place. As seen in cross-section (Fig. 3a), the fine fibrous perimysial network has become more congealed (P) with particulate material enmeshed within it. The muscle fibers are in tight contact with the endomysium (E). When viewed parallel to the fiber axis (Fig. 3b), the endomysium (E) in this instance appears nonfibrous and the perimysium (P) borders both sides of a muscle fiber bundle.

In Figure 4 are shown time-sequence light photomicrographs taken from a video recording of raw muscle tissue stressed parallel to the fiber axis. In 4a, initial stress is being applied; in 4b and 4c, the muscle fibers begin to rupture and thin strands begin to appear. As more stress is applied (4d), a number of fibers are definitely ruptured and the stranded material bridges the gap. The strands appear to originate from the perimysial connective tissue. In 4e and 4f, the muscle fibers have completely ruptured and only the strands remain intact. The force required to rupture the strands is approximately twice the force required to rupture the muscle fibers.

In Figure 5, both light microscopy and scanning electron microscopy observations of the same sample area in raw tissue are depicted. Figure 5a, taken from a video playback, shows the strands remaining before the complete rupture of the sample. The bright spots on the strands are moisture droplets reflecting the light. The same specimen carried through fixation and processed for the SEM is depicted in Figure 5b at higher magnification. Many of the strands are aligned in the direction of the stress. Some have ruptured and have sprung back to coil in a random fashion. The lower half of the micrograph shows

the ruptured ends of muscle fibers. The large fiber diameters of the strands as shown in Figure 5b give additional support for the perimysial origin of the strands, since the endomysial connective tissue appears to be composed of much finer fibers.

Efforts were made to identify these strands by staining techniques (Orcein, Weigert's, and Pollock's Trichrome stains). Positive results were obtained for collagen but no evidence was found for the presence of elastin. The strands, when viewed under polarized light, were seen to be birefringent, this result lending additional corroborative evidence for the collagenous nature of the strands.

Raw and heated (90°C) meat samples were stressed parallel and perpendicularly to the fiber axis almost to the point of rupture. Some samples did in fact break and these were discarded. In some other samples, especially for the parallel stress experiments, the overall structures remained intact, but portions of the myofibers had ruptured. The samples were removed in the stressed position, fixed in the clamping holder, carried through ethanol dehydration, and cryofractured.

In Figure 6a is shown interior surfaces of raw meat stressed parallel to the fiber axis. The strands appear taut and aligned in the direction of the applied stress. A similar orientation of the perimysium fibers is obtained with the heated muscle when stressed.

In Figure 6b is shown interior surfaces of heated meat stressed perpendicularly to the fiber axis. The rupture occurs first at the perimysium-endomysium junction. This is in contrast to parallel stressed tissue for which the muscle fibers rupture first. The muscle fibers in 6b appear undistorted, but the connective tissue has been aligned in the direction of the applied stress. Analogous results are obtained with the uncooked muscle.

For the parallel stressed samples, the aligning of the connective tissue in the direction of the applied stress as illustrated in the preceding micrographs begins to take place only after considerable stress is applied. At elongations of 50% of the rest length, for example, very little orienting of the con-
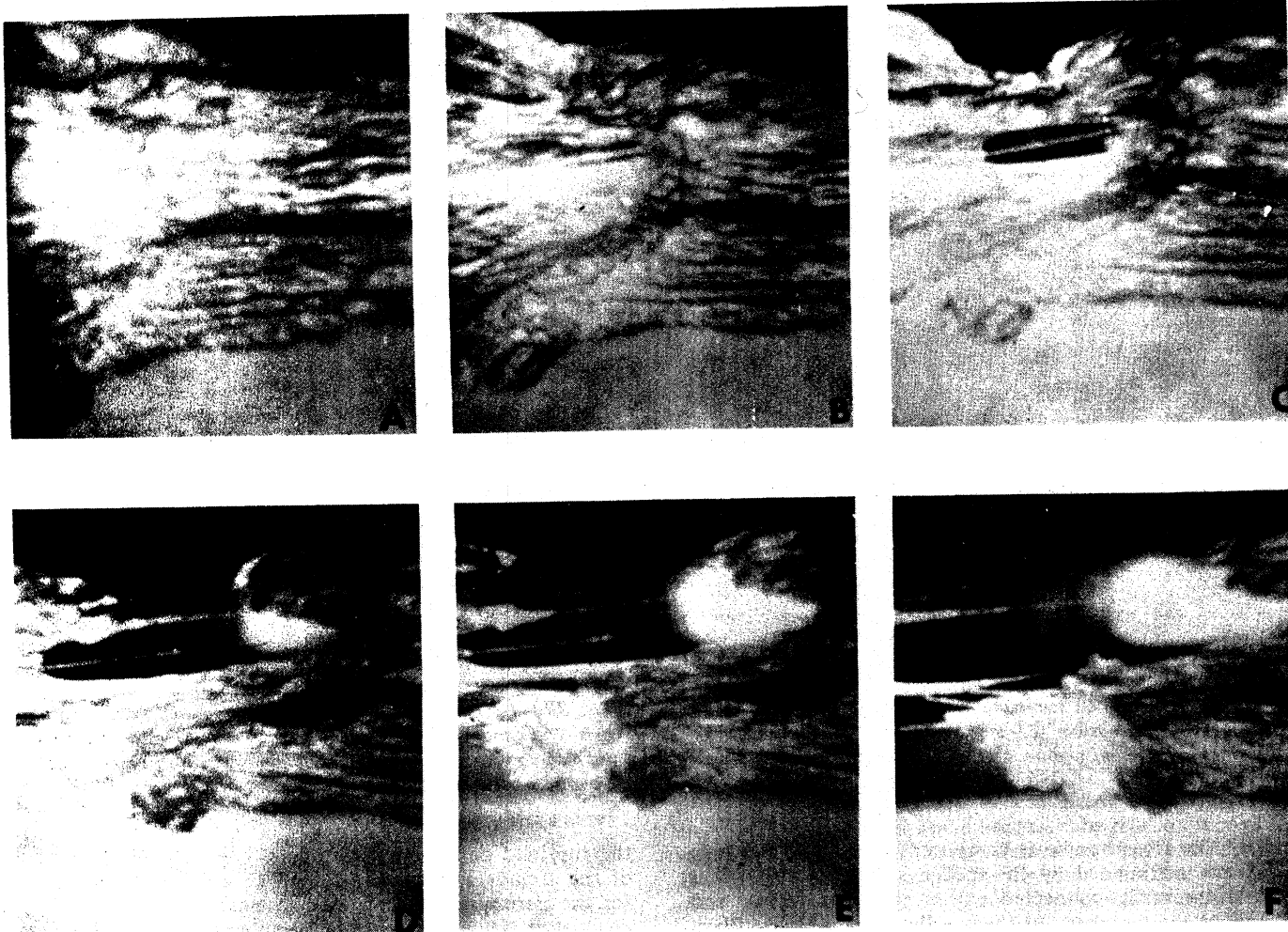
Fig. 4—A series of time sequence photographs taken from video tape of light microscopy of semitendinosus muscle under dynamic tensile stress. (Magnification ∿ 10X) The series of rings observed in the background of D, E, and F is the worm drive of the stage: (A, B) Muscle fibers begin to rupture; (C) Strand material is observed. (D, E) Network of strand material appears as more muscle fibers rupture; (F) Strand network intact after muscle fibers have ruptured.

nective tissue strands is evident. Consistent strand orientation is obtained only when the elongation reaches about 100%. On the other hand, for the perpendicularly stressed samples, consistent strand orientation is evident at about 50% elongation.

To explore in more detail the contribution of the perimysial connective tissue to meat structure, we carefully dissected the perimysium from raw and heated (90°C) meat tissue under a stereo microscope. Light microscopic examination of stained sections of this tissue showed some striated muscle and collagen of the perimysium. When viewed under polarized light the birefringence of unheated collagen fibers was quite evident. Small amounts of elastin were observed in association with occasional blood vessels. No elastin was observed in the perimysial tissue. In the heated perimysium, collagen fibers were found, though the birefringent appearance was somewhat less intense.

The isolated tissue, when observed in the scanning electron microscope, appeared as in Figure 7. In both instances, an interwoven fibrous network is observed, with fibers of various diameters similar to those of the intact tissue in Figure 3. The raw connective tissue, Figure 7a, displays an extensive network with a small amount of amorphous material with little structural detail. The perimysium from heated tissue, Figure 7b, still retains a fibrous appearance but with a significant amount of sharply defined particulates found on, around, and trapped within the fibrous network. Some of these particulates are

presumed to be formed by coagulation of the sarcoplasmic proteins. The fine fibers observed in the raw perimysium are not observed in the heated tissue, Figure 7b.

A portion of the isolated perimysium was dispersed in a Waring Blendor and examined with the transmission electron microscope. Figure 8a shows a network of dispersed fibrils displaying the banding pattern associated with collagen fibrils. Some electron dense material is also observed in this micrograph but is unidentifiable. In the heated perimysium (Fig. 8b), most of the sample appears as electron dense material in the transmission electron microscope. However, several collagen fibrils displaying typical banding pattern enmeshed within electron dense material can be observed. A rough estimate would indicate ∿ 10% of the observed specimen contained collagen fibrils showing the band pattern.

DISCUSSION

Although the minitensile stage, upon which the muscle tissue was stressed, is a crude device compared to Universal tensile testers, a rough estimate of rate of extension can be obtained from video tape playback. In our experiments, the rate of extension was approximately 1.0–1.5 cm/min, which was much slower than the 30 cm/min rate employed by Stanley et al. (1972) in their study on muscle tensile properties. We selected this slow extension rate so that we could pinpoint the sequence of events on the various muscle components and to

stop action as quickly as possible for observation in the scanning electron microscope.

Video playback of the stressing experiments shows that with the force applied parallel to the fiber axis (Fig. 4), the initial rupture occurs at the muscle fiber level, concomitant with the appearance of strand material. As the stress is increased, the muscle fibers completely rupture and only the stranded material remains intact. Approximately twice the force is necessary to rupture the strands as is required to rupture the muscle fibers.

On the other hand, when the force is applied perpendicularly to the fiber axis, the rupture occurs at the endomysial-perimysial connective tissue junction. The muscle fibers with their network of connecting sheaths remain relatively undisturbed.

Scanning electron microscopy of stressed but intact muscle shows the events taking place within the muscle structure prior to actual rupture. At elongations of 50% of rest length, very little change in the ultrastructure of the connective tissue is evident. At elongations of 100%, however, the individual fibers of the perimysial connective tissue are seen to align themselves in the direction of the applied stress. This is true for the raw as well as the cooked muscle (Fig. 6).

Since the major component of the perimysial connective tissue, collagen, is known to be relatively inextensible (Bailey, 1972), any apparent elasticity of the connective tissue must arise from reorienting individual fibers of the perimysial network. The perimysium of unstressed samples exhibits a multitude of randomly oriented fibers. Evidently, as the muscle tissue is elongated, the randomly oriented fibers become aligned in the direction of the applied stress (Stanley et al., 1972).

Eventually, as the muscle tissue is stretched, the fibers of the perimysium become fully aligned. Increased stress will cause them to rupture.

The orienting of the connective tissue fibers seems to take place for the cooked as well as the raw muscle. Heating to 90°C apparently does not completely destroy the fibrous nature of the perimysium. Even though the perimysium of the 90°C heated samples gives a more congealed appearance than that of the raw samples, well defined fibers remain and are subject to reorientation under stress.

SEM observations of the isolated perimysium yield results similar to those on the connective tissue in unstressed intact muscles for both raw and cooked samples. Fine fibrils, evident in the raw tissue, are absent in the cooked perimysium. However, the large connective tissue fibers persist even at the high temperatures despite the appearance of coagulated and amorphous material.

The transmission electron micrographs of the isolated perimysium reveal more fully the nature of the fibrous material. Figure 8a shows the typical banding pattern of native collagen in the uncooked material. For the heated sample, however, most of the fine collagen fibrils have been replaced by large amorphous and undefined masses (Fig. 8b). Nevertheless, some of the material retains the banding pattern of native collagen (roughly 10% of the total) which is indicative of the persistence of undenatured collagen even at 90°C.

This result is unexpected in view of the much lower shrink temperature of collagen. Intramuscular collagen has been reported to undergo shrinkage at 61–63°C as determined by differential scanning calorimetry (McClain et al., 1970; McClain, 1971) and has been found to undergo a precipitous drop in shear resistance in the interval 50–60°C attributable to collagen shrinkage (Draudt et al., 1964). Despite the uncertainty of the relationship of these measurements to those on intact collagen, it appears that intramuscular collagen undergoes a thermal transition comparable to that observed in skin and tendon collagen which is indicative of the unfolding of the collagen triple helix in the range about 60°C
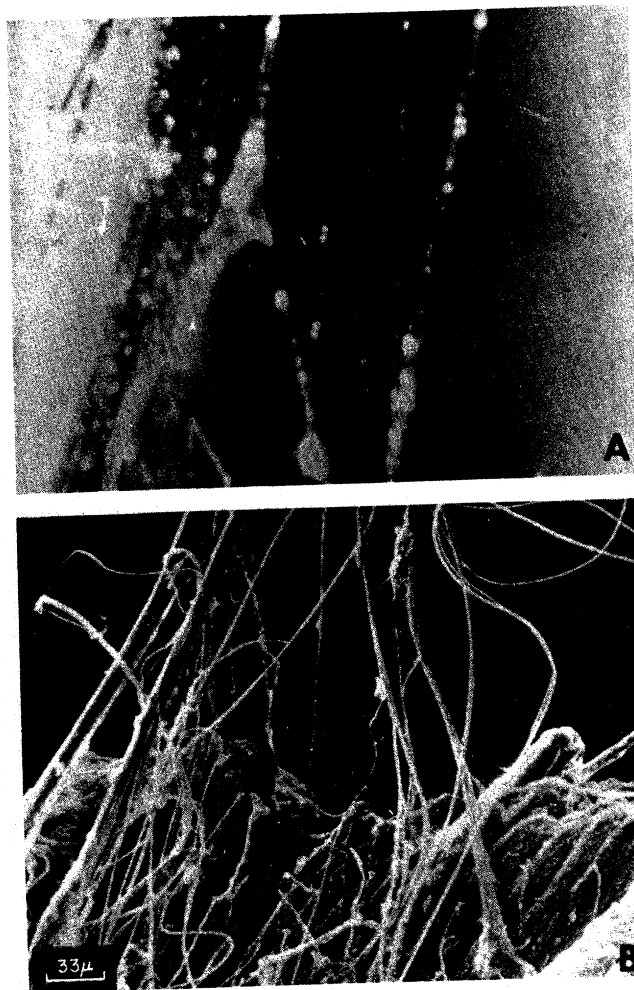

Fig. 5(a)—Light micrograph of strands under stress as observed on video playback. (B) Same area of (A) of the strands under stress and the ends of ruptured muscle fibers as observed in the scanning electron microscope.

(Ramachandran, 1967). By this reasoning, no collagen native structure should exist much above 60°C. Some native structure does remain at as high as 70°C as shown by Giles (1969) in transmission electron microscopy studies of 70°C heated muscle. However, the persistence of native structure in samples heated to 90°C for 45 min is very difficult to interpret.

There is evidence that intramuscular collagen is much more extensively cross-linked (some 10–14 times) than tendon collagen and that the cross-links are much more thermostable (Mohr and Bendall, 1969). It is possible that the collagen showing the banding pattern of the native structure represents a small portion of the collagen network that is sufficiently cross-linked to withstand these high temperatures without denaturing. The small quantity seen ($\sim$10%) would not be expected to alter significantly the shrink temperatures of the bulk of the material.

It is evident from these studies that the tensile properties of bovine muscle tissue depend very strongly on the nature, extent, and reaction to thermal stress of the perimysial connective tissue. These tensile properties, in turn, have a significant effect on both the subjective and objective evaluations of meat tenderness. Electron microscopy offers a powerful method of determining the relative roles of muscle tissue components in their reaction to a variety of mechanical, aging, and thermal stresses. Through a synthesis of the knowledge derived from studies of this kind extended to other stresses, an understanding of the factors which control meat tenderness as

Fig. 8(a)—TEM of dispersed perimysium from raw semitendinosus shows a random network of collagen fibrils with the characteristic band pattern. Some electron dense particulates are present.
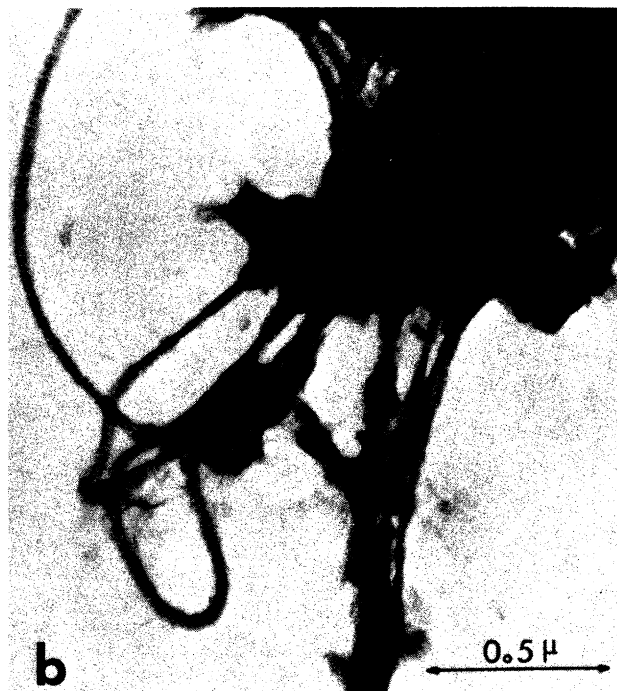

Fig. 8(b)—TEM of dispersed perimysium from heated (90°C) semitendinosus with some collagen fibrils remaining enmeshed in a large amount of amorphous material.

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